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APE1/Ref-1 Role in Redox Signaling: Translational Applications of Targeting the Redox Function of the DNA Repair/Redox Protein APE1/Ref-1

Mark R. Kelley[*,1,3], **Millie M. Georgiadis**[2], and **Melissa L. Fishel**[1,3]

[1]Department of Pediatrics (Section of Hematology/Oncology), Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA

[2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

[3]Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Abstract

The heterogeneity of most cancers diminishes the treatment effectiveness of many cancer-killing regimens. Thus, treatments that hold the most promise are ones that block multiple signaling pathways essential to cancer survival. One of the most promising proteins in that regard is APE1, whose reduction-oxidation activity influences multiple cancer survival mechanisms, including growth, proliferation, metastasis, angiogenesis, and stress responses. With the continued research using APE1 redox specific inhibitors alone or coupled with developing APE1 DNA repair inhibitors it will now be possible to further delineate the role of APE1 redox, repair and protein-protein interactions. Previously, use of siRNA or over expression approaches, while valuable, do not give a clear picture of the two major functions of APE1 since both techniques severely alter the cellular milieu. Additionally, use of the redox-specific APE1 inhibitor, APX3330, now makes it possible to study how inhibition of APE1's redox signaling can affect multiple tumor pathways and can potentiate the effectiveness of existing cancer regimens. Because APE1 is an upstream effector of VEGF, as well as other molecules that relate to angiogenesis and the tumor microenvironment, it is also being studied as a possible treatment for age-related macular degeneration and diabetic retinopathy. This paper reviews all of APE1's functions, while heavily focusing on its redox activities. It also discusses APE1's altered expression in many cancers and the therapeutic potential of selective inhibition of redox regulation, which is the subject of intense preclinical studies.

Keywords

Age related macular degeneration; base excision repair; redox angiogenesis; redox signaling; reduction-oxidation; tumor microenvironment

[*]Address correspondence to this author at the Betty and Earl Herr Professor in Pediatric Oncology Research, Professor, Departments of Biochemistry & Molecular Biology and Pharmacology & Toxicology, Associate Director, HB Wells Center for Pediatric Research, Associate Director of Basic Science Research, Indiana University Simon Cancer Center, Herman B Wells Center for Pediatric Research, Walther Hall - R3; Rm 528, 980 West Walnut, Indianapolis, IN 46202, USA; Tel: 317-274-2755; Fax: 317-274-8046, mkelley@iupui.edu.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest or any potential competing interests.

INTRODUCTION

Cancer is an accumulation of errors. So it follows that effective ways to arrest cancer should be to 1) block the first error in cancer's trajectory, or 2) block a pathway by which cancer sustains itself. Because the former approach has not materialized therapeutically to the extent originally hoped for (with the exception of treatments developed for chronic myelogenous leukemia), today's research focuses on upstream targets to block downstream events that could lead to uncontrolled cell growth. However, tumor cells, especially those in metastatic lesions, accumulate so many defects and acquire so many maladaptive processes that blocking a single pathway is unlikely to affect all the cells. Thus, the concept of targeting a single molecular pathway through inhibition appears to be efficacious in only limited patient populations. However, a single target that affects multiple pathways has the potential to produce a greater therapeutic effect within the heterogeneity of the tumor milieu. Theoretically, an advantage of finding such a target is that it could be clinically useful for numerous types of tumors.

Perhaps one of the most promising proteins being studied in this regard is APE1, named for its dual functionality (Fig. 1). APE1 is an apurinic/apyrimidinic endonuclease in the DNA base excision repair (BER) pathway, accounting for 95% of all such repairs of abasic (AP) sites in BER [1]—hence, the first part of its name: "APE1." BER is responsible for repairing DNA damaged by oxidative stress, alkylating agents, and ionizing radiation; and APE1 plays a critical role in such repair. APE1 also directly or indirectly influences other DNA repair activities, as noted in later in this article.

APE1 is the only known DNA repair protein that also functions as a reduction-oxidation regulator in mammals (Fig. 1). The first studies to uncover APE1's redox activity found that it was responsible for reducing transcription factor AP-1 (c-Jun/c-Fos), enhancing its DNA-binding activity. Therefore, this redox regulator was named "redox effector factor 1," or "Ref-1 [2, 3]. In this role, APE1 maintains a number of transcription factors in their active, reduced state, thereby influencing gene expression and maintaining genomic stability. Today, the complete name of this protein, APE1/Ref-1, is usually shortened to "APE1," as is done in this paper.

Emerging studies continue to expand the repertoire of APE1's activities. Its redox regulation influences stress responses, DNA repair, and other cellular functions, including angiogenesis, inflammation, and cell survival (Fig. 2). APE1 is so critical to maintenance of genomic stability that all attempts to generate a murine knockout model have proven to be lethal to embryos on days E5 to E9, and no viable cell lines completely deficient for APE1 have been established [4].

APE1's wide-ranging functions make it an attractive subject for translational research. But its multifunctionality also complicates its study, as, until now, it has been impossible to tease apart its repair functions from its redox activities and its other protein-protein interactions. Our identification and testing of selective small-molecule inhibitors of APE1's DNA repair and redox functions now make it possible to isolate how APE1 interacts in each of its domains, which paves the way for developing specific therapeutics that can enhance tumor cell killing while decreasing associated toxicities. APE1 is an upstream effector of multiple cancer survival mechanisms, so finding one protein whose inhibition would damage more than one survival mechanism holds great potential for the development of a novel, first-in-class molecularly targeted drug. This paper focuses on APE1's redox function and its selective inhibition, which is the subject of intense preclinical studies.

APE1'S VARIED FUNCTIONS

DNA Repair

APE1 is an essential base excision repair (BER) enzyme that orchestrates repair of DNA damage caused by oxidative stress, alkylating agents, and ionizing radiation. In this role, APE1 nicks the abasic site, then recruits other enzymes to remove the damaged bases, generate the correct bases, and ligate the repair (Fig. 3). APE1 also repairs damage caused by reactive oxygen species [5, 6]. In all cases, if that damage were left unrepaired, genetic instability [1] and/or cell death would result [7, 8].

APE1 is also thought to interact with a large number of other DNA repair-related proteins, including DNA glycosylases such as Ogg1 [9], DNA glycosylases, DNA polymerase β [10], X-ray cross-complementing-1 (XRCC1) [10–12], proliferating cell nuclear antigen (PCNA) [13], and flap endonuclease 1 (FEN1) [10, 13, 14] (Fig. 3B).

Other enzymatic repair activities associated with APE1 include 1) a 3′-repair diesterase or phosphatase activity [15], which is important for repair of DNA damaged by IR; 2) a 3′-5′ exonuclease activity, reported to play a role in the excision of deoxyribonucleoside analogs [16–19], and 3) a glycosylase-independent function in nucleotide incision repair, to generate 3′-hydroxyl and 5′-phosphate termini associated with oxidative damage to DNA [20, 21].

APE1 influences DNA repair activities beyond its direct repair function in the BER pathway (Figs. 2 and 4). The protein's redox regulation of transcription factors is one of several mechanisms that controls sequence-specific DNA binding, and thereby, gene expression. APE1's redox influence on p53, AP-1, and HIF-1α affects the DR, BER, HR, MMR and GGR pathways, as described in reviews by Kelley, Izumi, Mitra, and others [22–28].

Redox Regulation

Overview—APE1's duality in performing both endonuclease and redox activities is unique to mammals. For example, APE1 in zebrafish lacks any redox function, yet it is a fully active AP endonuclease. Thus, APE1's redox activity represents a novel redox component of signal transduction processes that regulate eukaryotic gene expression. APE1's reduction of disulfide bonds activates numerous transcription factors to enhance their DNA binding.

The redox and repair portions of APE1 are molecularly distinct physically and function completely independently of each other [29] (Fig. 1). Although the site for APE1's DNA repair activity has been fully characterized [30], the redox domain is poorly understood [31].

Mechanism of Redox Activity—How APE1 reduces transcription factors has not been fully elucidated [31, 32], as only one of its Cys residues (Cys65) has been identified as being critical for its redox function (Fig. 1). Typically in thiol-mediated redox reactions, one Cys residue of the redox factor serves as the nucleophilic Cys, which attacks the disulfide bond in another protein and forms a mixed disulfide bond, i.e. a disulfide bond between the redox factor and the target protein. The mixed disulfide bond is then resolved by attack of the "resolving" Cys residue within the redox factor. This results in the formation of a disulfide bond in the redox factor; the target protein is thereby reduced and the redox factor oxidized in this reaction. In other redox factors such as thioredoxin, the Cys residues required for the thiol-mediated redox reaction are found within a C-X-X-C motif. APE1 is a unique redox factor in that it lacks a C-X-X-C motif and has no Cys residues positioned appropriately to form a disulfide bond with Cys65 in the resolving step of the redox reaction. Cys65 is a buried residue located near the N-terminus on the first beta strand in the fold of a beta sheet in the protein's core. Cys 93 to date is the only other Cys residue that has been implicated in the redox activity of APE1 [31, 32]. We previously reported reduced redox activity for the

C93A APE1 mutant [31]. Interestingly, Cys 93 is also a buried residue located within the core of the protein on an adjacent beta strand but on opposite side of the beta sheet from Cys 65 (Fig. 1) [25]. The distance between Cys65 and Cys93 is 9 Å making it the closest Cys residue to Cys 65 in the reported crystal structures of APE1 [30, 31, 33, 34].

Our current hypothesis is that Cys65 serves as the nucleophilic Cys residue in the reduction of disulfide bonds in the transcription factors that are reduced by APE1 and that at least one other Cys residue, e.g. Cys 93, may serve as a resolving Cys residue. As Cys65 is not a solvent accessible residue, in order for it to serve as a nucleophilic Cys, a conformational change must occur in APE1. Recent data from Georgiadis, Gross, and Kelley laboratories suggests that APE1 can adopt an alternate, partially unfolded conformation and that a third Cys residue also plays a role in the redox activity (Su *et al*. and Georgiadis *et al*., manuscripts in preparation). In order for Cys93 or another Cys residue to resolve the mixed disulfide bond formed between APE1 and a transcription factor, a new disulfide bond would form in APE1 involving Cys65. It seems highly probable that disulfide bond formation involving Cys65 would occur in an alternate conformation of the APE1 as none of the Cys residues in the structure are appropriately positioned to form a disulfide bond with Cys65. Given the involvement of three Cys residues rather than just two as found in thioredoxin, the mechanism of disulfide bond reduction by APE1 is likely to involve additional steps and the formation of more than one disulfide bond.

There is precedence for the involvement of local unfolding in the mechanism of redox activity in peroxiredoxins, which catalyzes the reduction of intracellular hydrogen peroxide and other peroxides [35]. Although peroxiredoxins lack a C-X-X-C motif, two Cys residues are required for the redox activity. Within the fully-folded dimeric peroxiredoxin structure, the nucleophilic Cys residue is sequestered and the resolving Cys residue is located near the C-terminus, approximately 9 Å from the nucleophilic Cys. A local unfolding event involving the C-terminus of one subunit of the dimer places the nucleophilic Cys from one subunit proximal to the resolving Cys from the other subunit to form the active site that detoxifies H_2O_2 [36].

The Extent of APE1's Redox Activity—APE1 modulates the activity of a number of transcription factors involved in DNA repair and stress responses [6, 24, 26–28, 37], and other cellular functions [38–44] (Figs. 2 and 4). APE1 controls the redox status of both ubiquitous (AP-1, Egr-1, NF-κB, p53, CREB, HIF-1α) and tissue-specific transcription proteins (PEBP-2, Pax-5 and -8, TTF-1) [2, 38, 39, 41–47].

Some of the transcription factors under APE1's redox influence include those involved in cancer promotion and progression, notably AP-1 (Fos/Jun), NF-κB, PAX, HIF-1α, HLF, p53 and others [6, 24–27, 31, 37, 48]. The tumor milieu of pancreatic cancer is a good example of how multiple pathways can be affected by a single target to produce a greater therapeutic effect (Figs. 4 and 5). Four of the transcription factors that APE1 regulates (NF-κB, AP-1, Stat3 and HIF-1α [25, 37, 49, 50]) have been implicated in pancreatic cancer progression, resistance to therapy, and metastatic potential [51, 52]. A recent review indicates that those four transcription factors are key regulators of multiple signals in pancreatic cancer and provides strong evidence for investigating the effects of targeting transcription factors to kill pancreatic cancer cells [53]. Therefore, blocking APE1 redox function could have a significant therapeutic effect and reveal more about which transcription factors are crucial to tumor survival in pancreatic cancer (Figs. 4 and 5).

Not only do these four transcription factors regulate signaling within the tumor, but they also influence the tumor microenvironment [27, 54], as downstream effectors of these transcription factors are tightly linked to cancer cell growth and metastasis [53]. Therefore,

we believe that APE1's redox co-activation of transcription factors is worth pursuing, as it is a novel and emerging area of study.

Other targets of APE1 redox signaling are also of great interest. In particular, p53, AP-1, and HIF-1α exert modulatory effects on many pathways, which make APE1 inhibition even more important as a potential clinical therapeutic (Table 1). As noted earlier, the more pathways that can be modulated, the more likely one is to affect more cancer cells in the heterogeneity of a tumor. Those three transcription factors and their interactions with APE1 are discussed further on the following pages.

Analysis of APE1's effector action on these proteins underscores additional unique features of APE1. First, APE1 acts upon transcription factors that are unrelated multi-domain proteins. APE1 does not recognize any common structural motif when acting upon them, and their redox-regulated DNA-binding domains are structurally distinct: p53 is a single immunoglobulin-like domain; NF-κB is a dimer of two immunoglobulin-like subunits; AP-1 is a heterodimeric bZip family protein, and HIF-1α is a basic helix-loop-helix domain. Second, the nature of the reactions varies. APE1 copurifies with AP-1 [2] in a stable reaction. In contrast, APE1's interaction with p53 is transient.

p53: p53 is a transcription regulatory protein that helps preserve genomic integrity by its participation in stress-response pathways and DNA repair pathways [42, 55–57]. As such, it is often called "the guardian of the genome" [58]—and with good reason: more than half of all cancers contain mutant or inactive p53.

This regulatory protein is an important tumor suppressor with multiple strategies for maintaining genomic stability. To avoid mutability, p53 either arrests the cell cycle or induces apoptosis, based on cellular conditions. For example, hypoxia in tumor cells prompts p53 to induce apoptosis [59]. In other circumstances when p53 senses DNA damage, it can halt the cell cycle at G_1 by blocking Cdk2 activity, allowing sufficient time for DNA repairs to be completed [60]. The far-reaching influence of p53 also includes complex interactions with other proteins and effector genes.

The earliest studies of p53's redox influence were based on the finding that oxidized p53 has little binding affinity for DNA [61]. This led to the discovery that APE1 was responsible for reducing wild-type p53, thus enhancing its DNA-binding activity [59].

Although the mechanisms by which APE1 enhances p53 activity are still being discovered, they appear to include two coordinated activities: a redox-dependent activation of the DNA-binding domain (through direct reduction of a disulfide bond), as well as a redox-independent mechanism [59]. APE1's reduction of p53 [59] is essential, but not sufficient, for optimizing p53's DNA-binding function [59]. Recent evidence shows that APE1 also helps p53 obtain its optimal DNA-binding configuration by interacting with p53's C-terminal regulatory domain in a redox-independent manner, facilitating tetramerization of p53. Thus, APE1 appears to enhance sequence-specific DNA-binding activity through two independent, coordinated mechanisms [62].

Redox signaling involving p53 depends upon APE1, plus a general redox factor as well. Thioredoxin (Trx) enhances APE1's stimulation of p53-dependent expression of p21, which suggests a link between Trx and APE1 in cellular response to oxidative stress [44].

Because p53 influences many DNA repair pathways and APE1 constitutively influences p53, APE1 contributes to p53's many DNA repair activities (Table 1). p53's regulation of DNA repair is complex, involving both transactivation-dependent and -independent mechanisms that affect the HR, BER, NER, MMR and NHEJ pathways [56]. The

mechanism of action depends on the genotoxic stress present and may also depend on the cell line involved [25, 27, 28].

For example, in the BER pathway, recent studies of p53 and APE1 in colorectal cancer cell lines reveal a system of checks and balances between the two, maintaining equilibrium between p53's proapoptopic activity and APE1's pro-survival activity. In HCT116 cells, Zaky *et al*. noted that wild-type p53 (but not mutant p53) downregulated APE1 by indirect recruitment of its promoter, possibly blocking binding of SP1 [58]. In the GGR sub-pathway of NER, p53 plays a role, but only after cells are exposed to sublethal radiation and arresting cells at G_1, to allow time for DNA repair before the cycle progresses to the S phase [63, 64]. PCNA, a procressivity factor in the NER pathway, is also under p53 regulation [65]. In the MMR pathway MSH2, MLH1, and PMS2 are under p53 regulation, similar to the way that DDB2 and XPC are in NER [66, 67].

In the HR pathway, p53 inhibits HR through repression of RAD51 expression [68]. While in NHEJ, p53 downregulates expression of WRN and RecQ4, two RecQ helicases [69, 70]. However, p53 exerts differential regulation of AGT in the **DR** pathway [71, 72], depending on the type of cellular stress present. Contributing to this complexity is the fact that AGT also appears to be regulated by NF-κB, a transcription factor mediating immune and inflammatory responses [73]—and NF-κB is under APE1 redox control.

All of these studies point to the possible clinical relevance of redox-controlled DNA repair responses through p53. If reduced p53 is required to bind DNA and either activate or repress the transcription of DNA repair genes, then it follows that APE1 plays an important role not only in the redox modulation of p53, but also in the regulation of DNA repair.

AP-1: Activator protein-1 (AP-1), also referred to as tetrade-canoylphorbol-13-acetate (TPA)-responsive elements, constitutes a family of proteins that recognize AP-1 sites. AP-1 mediates cellular proliferation, differentiation, apoptosis, and transformation; it also responds to environmental changes including growth factor signals, stress, and radiation [74]. AP-1 is induced rapidly in response to many cellular stimuli, and its activation regulates the expression of several proteins involved in multiple DNA repair pathways.

Although it is diverse, the AP-1 family is a group of structurally and functionally related basic leucine zipper proteins (bZIP) that intermix to form heterodimeric sequence-specific DNA-binding proteins. These include Jun proteins (c-Jun, JunB, and JunD), Fos proteins (c-Fos, FosB, Fra-1 and Fra-2), and some ATF family members (ATFa, ATF-2, and ATF-3) [74].

Studies to determine the nuclear factor responsible for reducing AP-1 (c-June/c-Fos) led to the initial identification of APE1's redox activity [2, 75]. APE1's oxidation/reduction of conserved Cys residues within the basic DNA-binding domains of c-Jun and c-Fos enables them to dimerize, thus enhancing AP-1's DNA-binding ability [76]. Since then, Trx has also been identified as a factor modulating transcriptional activation of AP-1 through its reduction of APE1 [40].

Because AP-1 must be reduced to bind to its target sequence, redox control of this protein has significant implications. APE1 appears to be the key point of control for regulating the DNA-binding activity of AP-1, thus modulating the expression of various DNA repair proteins (Table 1).

HIF-1α: Hypoxia inducible factor-1 (HIF-1), which responds to low-oxygen conditions in the cell, is under scrutiny for its role in the tumor microenvironment, as many cancers

flourish in hypoxic conditions [77, 78]. Also, increasing evidence reveals that hypoxic stress in the tumor microenvironment can cause genetic instability in cancer cells [78, 79].

HIF-1 is a heterodimeric transcription factor comprised of two subunits; the HIF-1α subunit is crucial for regulating cellular response to hypoxia and is frequently overexpressed in human cancers. Cellular hypoxia causes HIF-1α to translocate to the nucleus and dimerize with HIF-1β, forming HIF-1. APE1 has been implicated in providing redox-dependent stabilization of HIF-1α so that it can activate HIF-1 [80]. Redox signaling also regulates the DNA-binding activity of HIF-1, which, along with coactivators, binds hypoxia response elements (HRE) within promoters and regulates the expression of their downstream genes, including vascular endothelial growth factor (VEGF) miR210 and CA-9 (Fig. 4) [81, 82]. Thus, APE1 facilitates the formation of the hypoxia-inducible transcriptional complex that includes HIF-1 and transcriptional coactivators p300 and CREB. More recently, HIF-1α has been shown to play a role in down-regulating mRNA and APE1 levels under hypoxic conditions in human microvascular endothelial cells [83]. Thus, HIF-1α regulates expression levels of APE1 and is itself regulated by APE1 (Figs. 4 and 5).

Hypoxia downregulates the expression of key genes within the HR and MMR DNA repair pathways [78, 79, 84–91]. Interactions between HIF-1α and relevant DNA-repair genes are still being determined, but some studies allude to a system of checks and balances between HIF-1α and p53 in DNA repair [89]. Because APE1 is required for redox regulation of HIF-1α, APE1 is an upstream regulator of DNA repair genes controlled by HIF-1α.

Other APE1 Protein-Protein Interactions

APE1 also exhibits redox-independent transcriptional regulatory functions that are subject to post-translational modifications, including phosphorylation, acetylation, and nitrosation. The functional roles of these modifications are still being elucidated. For example, acetylation of APE may influence its subcellular localization or trans-acting functions [92]. Review articles by Bhakat and others describe these implications further [24, 92].

Additional functions have been ascribed to APE1. For example, it is involved in NK-cell-mediated killing through granzyme A (GzmA) [93, 94], prevents oxidative stress by negatively regulating Rac1/GTPase activity [95], regulates endothelial NO production and vascular tone [96] and suppresses the activation of PARP1 during the repair of oxidative DNA damage [97]. Other findings include an inverse relationship between APE1 and Bcl2 levels [98] and its negative regulation of the parathyroid hormone gene and PTEN activator [99–103].

OTHER GLOBAL INFLUENCES OF APE1

Angiogenesis

One of the most exciting new discoveries about APE1 is its redox role in angiogenesis. APE1's redox function regulates several transcription factors (HIF-1α NF-κB, and AP-1) that are known to contribute to angiogenesis (Figs. 2, 4 and 5). (For review, see Maulik *et al.* [104]).

Recent *in vitro* studies suggest that APE1 redox activity is required for retinal vascular endothelial cells (RVECs) to proliferate and form tubules [27] Additionally, these studies demonstrate thatAPE1 is highly expressed in murine retinas, choroid/retinal pigment epithelium (RPE), RVECs, and retinal progenitor cells (RPCs). This suggests that APE1 redox activity is required for efficient retinal endothelial cell proliferation, migration, and tube formation [27].

These studies also showed that, using a neovascularization model with *vldlr*$^{-/-}$ knockout mice, a single intravitreal injection of APX3330 reduced subretinal neovascularization (SNV) in the eyes of *vldlr*$^{-/-}$ mice [105, 106], strongly supporting a possible role for APE1 in retinal vascularization. The authors also demonstrated that, when combined with bevacizumab *in vitro*, APX3330 strongly enhanced the observed anti-angiogenic effect (manuscript in preparation [107]).

Although such studies with eye disease are only beginning, they demonstrate another important therapeutic avenue by which to apply APE1 redox signaling. For example, recent studies suggest that APX3330 or some of its newly created analogs [108, 109] have potential as therapeutic agents for treating age-related macular degeneration (AMD). While anti-VEGF treatment is the current standard of care for wet AMD, the cost of monthly treatments for an indefinite time is expensive and often does not improve patients' vision. Thus, APX3330 may be especially useful for treating AMD that is refractory to anti-VEGF agents. The efficacy of combining APX3330 with anti-VEGF agents is also being explored for treating AMD [107].

These and other studies of APE1's redox activity in normal endothelial cells demonstrate another arena in which APE1 influences multiple pathways. APE1 helps 1) maintain cell differentiation, 2) modulate vascular tone by regulating NO levels [110], 3) enable differentiation of angiogenic progenitor cells and 4) prevent apoptosis by repressing pro-apoptotic TNFα signaling and upregulating pro-survival NF-κB signaling. When combined with *in vitro* and preclinical studies of APE1's inhibition, we can begin to infer what APE1's influence may be in the tumor microenvironment—and the potential clinical utility of a redox-specific APE1 inhibitor.

Tumors die without ready access to a blood supply. Migration of endothelial progenitor cells to the tumor microenvironment is essential for neoangiogenesis. Preclinical utility studies of APX3330 demonstrate that it inhibits growth of pancreatic cancer cell lines [111] as well as pancreatic cancer-associated endothelial (PCECs) and endothelial progenitor cells [110] (Figs. 4 and 5).

Additional studies of APX3330's effect on human bone marrow cells, pancreatic cancer cells and human umbilical vein endothelial cells show that APX3330 can reduce tumor endothelial VEGF secretion—and, at the same time, down-regulate levels of the cognate receptor Flk-1/KDR on PCECs, blocking a potentially critical angiogenic ligand-receptor interaction in the tumor microenvironment. In addition, APX3330 can block the differentiation of bone-marrow hemangioblasts [110].

We already noted APE1's redox regulation of HIF-1α, which mediates stress responses to hypoxia. Intracellular hypoxia is a feature of many cancers, including pancreatic and prostate carcinomas. APX3330 inhibits HIF-1's DNA-binding activity, which is consistent with APX3330's ability to inhibit APE1's redox functions. Thus, decreasing APE1 activity through chemical inhibition induces endothelial cell growth arrest *via* a signaling cascade upstream from HIF-1α [92].

Inflammation

Long-standing inflammation is a risk factor for tumorigenesis. For example, chromosomal instabilities can be detected even in early-stage dysplastic ulcerative colitis (UC) tissue [112]. When patients live with UC for more than 10 years, they have a 20- to 30-fold increase in the risk of developing colorectal cancer.

Inflammation caused by sources such as ROS and toxic agents transiently increases intracellular APE1 [6, 24, 25]. When prolonged intracellular stress continually stimulates APE1, the chance for genomic instability increases, as APE1's endonuclease activity produces a cytotoxic DNA repair intermediate: an abasic site [26]. If DNA repair stalls or is performed incorrectly, this can lead to microsatellite instability (MSI)—which is present in the aforementioned dysplastic colon cells.

In addition, inflamed areas of UC lesions contain significantly greater amounts of APE1 and another BER enzyme the adenine alkylguanine glycosylase (AAG), than non-inflamed tissues, suggesting that long-term [111] adaptive increases in these proteins contribute to the production of microsatellite instabilities (MSI). Many other sporadic tumors also contain MSI; overexpression of AAG and APE1 is likely just one example of how long-term dysregulation of a DNA repair pathway can contribute to tumorigenicity [113].

As more data are collected, it is becoming apparent that APE1's redox function is more involved in cell growth and angiogenesis activities, while its endonuclease repair activity is more highly attuned to cellular death [27, 110, 111, 114] In other words, APE1's DNA repair function helps prevent apoptosis triggers [24, 48, 114–116] in contrast, APE1's protein-to-protein interactions help promote cellular proliferation.

APE1 IN THE TUMOR MICROENVIRONMENT

We have barely scratched the surface in elucidating how APE1 behaves in the tumor microenvironment. Changes in intracellular distribution of APE1 have been noted in lung, ovarian, thyroid, and breast cancers and are associated with tumor aggressiveness and poorer prognosis [37, 117]. Higher nuclear and cytoplasmic expression of APE1 could be a response to oxidative stress, but the true etiology of this observation is yet unknown. Poorly differentiated hepatocellular carcinomas have a three-fold higher expression of APE1 compared to normal cells [118].

A paucity of information currently exists regarding DNA repair in the tumor microenvironment, except that it is abnormal. However, we can infer several key points from DNA repair in normal cells. First, if DNA repair is imperfect or incomplete, it can result in genomically unstable DNA damage, which is a key step for cancer initiation and progression [119, 120]. Second, partial backup systems or alternate routes exist for DNA repair. Tumors are typically defective or deficient in one DNA repair pathway, but they can compensate by using other repair mechanisms. Crossover, interaction and compensation within and among DNA repair pathways often allow cancer cells to avoid apoptosis [121]. For example, cancer cells deficient in one or more proteins of the HR pathway may compensate by repairing double-stranded breaks through the NHEJ or BER pathway [74, 122–124].

A tumor's DNA repair abilities are directly related to its intrinsic or acquired cellular resistance to clinical DNA-damaging agents and its unrestricted cellular proliferation [125–128]. So, in theory, a highly effective therapeutic agent would block one or more specific proteins in DNA damage repair pathways to eliminate the tumor's main means of survival. Because such a therapeutic would exploit a feature that makes cancer cells highly mutable, the therapeutic should not harm normal cells [121]. Therefore, inhibiting specific proteins in DNA damage repair pathways is a promising strategy for developing molecularly targeted cancer treatments [125–129]. This approach is taken to the next level when inhibition of one protein can effectively block the activities of multiple repair pathways or downstream multiple targets.

A clear example of this concept is found in the numerous studies of AP-1's influence on DNA repair proteins (Table 1). Research has uncovered more than 20 genes whose promoters are bound by AP-1 following cisplatin treatment. The proteins that AP-1 regulates in repairing DNA-cisplatin adducts include RAD23B, XPA, ERCC3, XPF, ERCC1 and ERCC3 in the NER pathway and MSH2, MSH6, MLH1 and PMS2 in the MMR pathway. Phosphorylated c-Jun or ATF2 activates these proteins to increase their DNA binding capacity [25, 27]. Because APE1 reduces AP-1 to its active form, inhibition of APE1 would have a profound effect on inhibiting those signaling pathways and could, effectively, enhance cisplatin treatment of the tumor.

Because cancer promotion and deregulated cellular growth are aided by deficient DNA repair pathways, it may sound counterintuitive to want to inhibit DNA repair pathways. In addition, some people debate the wisdom of inhibiting essential DNA repair enzymes [26, 28, 37, 130, 131]. However, a fine but distinct line exists between induction of DNA damage and its efficient repair [125, 129]. Inhibiting specific DNA repair proteins can selectively sensitize cancer cells to chemotherapeutic agents [128, 129]. Thus, developing DNA repair inhibitors targeted to the appropriate repair molecule to use as adjuncts of current chemotherapy is an area of great clinical interest as evidenced by the recent activity around PARP inhibitors [132, 133].

Redox control of transcription factors in the cancer tumor milieu is an area unexplored to date. However, this is an important area of investigation, as redox regulation may contribute to cancer cells' inherent resistance to chemotherapy. At the same time, study of redox activities may uncover viable new molecular targets for chemotherapeutic agents. In the redox arena, APE1 is the most significant contributor of signaling to downstream transcription factors.

MODULATING APE1'S ACTIVITIES AS A CANCER THERAPEUTIC APPROACH

In general, elevated APE1 levels are associated with aggressive proliferation, high angiogenesis, increased resistance to therapeutic agents, incomplete therapeutic response, shorter time to progression, poor prognosis and poorer survival rates [6, 134–139]. Altered or elevated levels of APE1 have been observed in breast and ovarian cancers, gliomas, sarcomas (osteosarcomas, rhabdomyosarcomas) and multiple myelomas, among others [61, 62, 89, 140–142]. Given that APE1 expression appears to be linked to chemoresistance and APE1 is altered in a variety of cancers as well as in multiple myeloma [143], developing a modulator of its activity can play an important role in the treatment of these diseases (Fig. 6).

Inhibition of APE1's redox activity in promoting cancer cell death and inhibition of tumor cell growth is a novel approach that has been unexplored to date, but the anti-cancer potential of an APE1 redox inhibitor is great. Transcription factors including HIF-1α, p53, NF-κB, CREB, and AP-1 have all been implicated in major aspects of cancer survival, including angiogenesis and tumor promotion and progression [53, 54]. Inhibiting APE1's redox activity would render these transcription factors unable to bind to DNA, thereby halting tumor cell signaling of angiogenesis and uncontrolled growth (Figs. 4–6).

Ample evidence exists that decreasing APE1 levels can block tumor cell growth and increase cellular sensitivity to DNA-damaging agents [114, 138, 144–146]. However, it is important to distinguish and characterize which of APE1's function(s) are involved in different biological events—and what functions vary between normal versus pathologic cells.

Despite the discovery of APE1's redox activity more than 10 years ago, few data exist describing how inhibition of the redox activity of APE1 may affect cancer cells' response to chemotherapy. Furthermore, the relative importance of APE1's redox vs. DNA repair function in cancer is unknown. Efforts to determine the differential therapeutic effects of inhibiting one or the other are ongoing.

Exploration of novel targets clearly merits pursuit, particularly in cancers for which few treatments exist or current treatments are largely ineffective. Most small-molecule inhibitors in development today target specific DNA repair enzymes. Those showing the most promise clinically include MGMT (AGT), poly ADP-ribose polymerase (PARP1), ataxia-telangiectasia mutated kinase (ATM kinase), APE1 (its endonuclease function) and DNA PKcs [37, 130, 131].

Small-molecule inhibitors that can attenuate one or more of APE1's functions offer potential for reversing drug resistance [26, 92, 108, 109, 121, 147, 148]. Finding specific small-molecule inhibitors that can block either of APE1's repair or redox functions, but not both, will help determine how to best modify its function in treating different diseases. Inhibiting APE1's redox influence on multiple pathways appears to hold great promise for helping alleviate chemoresistance.

The Redox Activity of APE1 as a Target in Cancer Treatment

Pharmacologic inhibition of NF-κB is an expanding area of drug development because NF-κB is activated following chemotherapy and radiation (reviewed in [149]). This activation, particularly after gemcitabine treatment [150, 151], can cause treatment resistance. By inhibiting APE1's redox activity, we and others can decrease the ability of NF-κB to bind to DNA [2, 6, 152], thus increasing the cancer cells' response to chemotherapeutics in cases where NF-κB is known to contribute to resistance.

NF-κB is just one of many transcription factors that would be unable to bind to DNA following treatment with a redox-specific APE1 inhibitor such as APX3330 [153–155]. Other transcription factors affected similarly would include AP-1 (Fos/Jun), HIF-1α, HLF, CREB, p53, and others (also reviewed in [6, 25, 27]). Studies to identify and use current and novel APE1 redox inhibitors are ongoing in our laboratory (manuscript in preparation).

Another likely pathway to exploit through inhibition of APE1's redox function is that of VEGF regulation *via* HIF-1α [156]. The regulation of genes involved in tumor progression, angiogenesis, and vasodilation by HIF-1α suggests that inhibition of its binding to DNA in a tumor could have clinical potential [157].

APE1 Redox Inhibitors Being Studied

Naturally Occurring Compounds—Two natural compounds, resveratrol and soy isoflavones, have been reported to modulate APE1 redox signaling and are being studied for possible clinical utility with cancers. Computer modeling of resveratrol, a component of grapes and red wine [158], showed it could bind the redox domain of APE1 [158]. However, these findings have not been substantiated as resveratrol does not appear to be effective as either a DNA repair or APE1 redox inhibitor in other studies and does not appear to be APE1-specific [27]. Also, in our studies of resveratrol, we were unable to inhibit APE1's redox or DNA-repair function with resveratrol at reasonable micromolar levels, including levels 5 times higher than used previously [27]. This finding is significant, as resveratrol has multiple reported activities with one being APE1 redox inhibition [158, 159].

Soy isoflavones, a component of soybeans, are being investigated for their potential use against prostate cancer and possible treatment of coronary heart disease and menopause

[152]. *In vitro* and *in vivo*, soy isoflavones combined with radiation decreases the survival of prostate cancer cells compared to cells treated with radiation alone, and the authors implied that soy isoflavones decrease the amount of APE1 available to reduce NF-κB, thereby causingthe increased sensitivity to radiation [152]. It is yet unclear whether APE1's redox or DNA repair function or both are affected by the addition of soy isoflavones. Regardless of which function may contribute more to this effect, utilizing soy isoflavones to block the redox signaling through APE1 and NF-κB dramatically increased prostate cancer cells' sensitivity to radiation. While these findings are exciting, more studies will need to be performed to confirm this initial finding.

APX3330 and its Analogs—APX3330 ([(2E)-3-[5-(2, 3dimethoxy-6-methyl-1,4-benzoquinolyl)]-2-nonyl-2-propenoic acid]) is a quinone compound with clinical potential as a redox inhibitor of APE1 (Figs. 4 and 6). Our earlier studies demonstrated APX3330's specificity in selectively blocking APE1's ability to convert a variety of transcription factors from an oxidized to a reduced state, effectively preventing certain genes from being "switched on" [27, 108]. A number of studies have demonstrated the specificity of APX3330 for APE1 [110, 111, 155, 160]. Numerous studies of ours and others have verified that APX3330 selectively inhibits NF-κB–mediated gene expression without affecting degradation of IkB alpha, translocation of NF-κB into the nucleus, and post-translational modification of NF-κB [153]. APX3330 inhibited the redox-sensitive DNA binding activity of NF-κB [2, 153, 154]. In addition, Matrigel assays and aortic ring formation assays demonstrated that APX3330 blocked ECFC angiogenesis *via* APE1 inhibition both *in vitro* and *in vivo* [25, 27, 110, 111]; APX3330 also has been shown to increase the effects of hypoxia [111], and affect pancreatic tumor xenograft cell growth [161].

After synthesizing a number of novel APX3330 analogs [108, 109], we have data on three of the most promising compounds. In a series of experiments using EMSA as described previously [27, 108], we determined that the analogs had redox blocking effects similar to that observed for APX3330. Using AP-1 as the transcription factor target complex, we determined that the IC_{50} for these analogs were at least 10 times lower than that of APX3330 [108, 109]. Cell-based transactivation assays using the ovarian cancer cell line SKOV-3X were performed to determine if APX3330 and its three lead analogs decreased NF-kB's ability to bind to its promoter and drive luciferase expression. All three analogs demonstrated more than three times the dose-dependent effect that APX3330 had on NF-κB activation in this assay [108, 109]. In cell growth assays using the ovarian SKOV-3X and Hey-C2 cell lines, we demonstrated that two of the analogs had up to a six-fold greater inhibitory effect than APX3330 did, supporting our previous observation that APE1's redox function affects cell growth—and blocking this function arrests, but does not kill cells [161]. TUNEL assays on ECFCs confirmed that two of the three analogs inhibited cell growth and tube formation through means other than apoptosis [27]. Studies on normal dividing cells and neuronal cells (data not shown) demonstrated that APE1's redox function had no effect on cell survival [60, 144, 162, 163]. Experiments are ongoing in our laboratory and in collaboration with Dr. Millie Georgiadis to determine the exact nature of the binding of APX3330 to APE1, and we anticipate characterizing a novel redox mechanism based on further analysis of the interaction of APX3330 with APE1.

Progress to Date: Our data, and others (see recent reviews [24, 25, 27, 116]) support the use of APE1 redox inhibitors in multiple cancer cell lines as an effective approach and confirm that blocking APE1's redox function blocks the DNA binding of numerous important transcription factors [25, 27, 110, 111]. Additional ongoing studies in our laboratory in this area include xenograft models and the development of more potent and sub-micromolar APE1 redox inhibitors. Further studies of APX3330 and analogs with clinical utility are being pursued to identify a definitive lead compound with the greatest

clinical therapeutic potential. We are ardently pursuing the use of APE1 repair and/or redox inhibitors as single-agent treatments or as adjuncts to standard chemotherapy and ionizing radiation regimens.

Challenges Remaining: A number of challenges remain before an APE1 redox inhibitor can move from bench to bedside. As mentioned, we are in the process of finalizing a lead candidate that is effective at sub-micromolar concentrations with strong APE1 specificity and specific APE1 redox inhibition. A number of candidates have been identified and more are being studied. These new analogs will also be useful for characterizing APE1's redox functions in cell and animal models. Data to date show the potential for a redox-specific APE1 inhibitor in treating AMD and a variety of cancers, and may have other indications for any area involving APE1 and the tumor microenvironment (Fig. 6).

CONCLUSIONS

APE1 plays a critical role in cellular redox activities—not only through its presumed control of DNA-repair proteins, but also through its redox signaling activities. Here we discuss emerging data demonstrating APE1's involvement in redox regulation associated with DNA repair, angiogenesis, inflammatory responses, and its potential role in the tumor microenvironment. Emerging data show that APE1 plays functional roles in tumor cell survival and proliferation and demonstrates altered expression primarily at the protein or subcellular localization level. Given APE1's 1) altered levels of expression in a variety of cancers [134–136, 164–167] and 2) its influence on a variety of important cancer signaling pathways ([25, 27, 53] and references therein), abundant evidence supports further study of APE1 as a viable target for cancer therapeutics. We propose that chemically knocking down or blocking the redox function of APE1 *via* selective inhibition can lead to greater tumor growth inhibition, alterations of the tumor microenvironment including inflammation as well as enhanced tumor cell killing, underscoring APE1's importance as a prime candidate for targeted molecular therapy.

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ABBREVIATIONS

8-oxoG	8 oxoguanine
AAG	alkyladenine DNA glycosylase
AGT	O-6-alkylguanine-DNA methyltransferase
AMD	age-related macular degeneration
Ang II	angiotension II
AP	apurinic/apyrimidinic
AP-1	activator protein 1
APE1	apurinic/apyrmidinic endonuclease 1
ATF	activating transcription factor
ATFa	activating transcription factor a

ATF2	activating transcription factor 2
ATF3	activating transcription factor 3
ATF4	activating transcription factor 4
ATM	ataxia telangiectasia mutated protein
BER	base excision repair
BKca	Large-conductance Ca^{2+}-activated K^{+} channels
BLM	Bloom's syndrome gene product
BRCA1	breast cancer 1, early onset
BRCA2	breast cancer 2, early onset
BSO	buthioninesulfoximine
CD40L	CD40 ligand
Cdk2	cyclin-dependent kinase 2
CHO	Chinese hamster ovary cells
CREB	cyclic AMP response-elementbindingprotein
CSA	Cockayne's syndrome protein A
CSB	Cockayne's syndrome protein B
CTL	cytotoxic T lymphocyte
DDB1	DNA damage binding protein 1
DDB2	DNA damage binding protein 2
DNA-PK_{cs}	DNA-dependent kinase catalytic subunit
DSBs	double-strand breaks
DR	direct repair
dRP	deoxyribophosphate
dRPase	deoxyribophosphodiesterase
APX3330	2E-3-[5-(2, 3 dimethoxy-6-methyl-1,4-benzoquinolyl)]-2-nonyl-2-propenoic acid
EGR-1	early growth response protein 1
ERCC1	excision repair cross-complementation group 1
ERCC3	excision repair cross-complementation group 3
ERCC5	excision repair cross-complementation group 5
$FADH_2$	flavin adenine dinucleotide dehydrogenase
FEN1	flap endonuclease 1
Flk-1/KDR	fetal liver kinase 1/kinase insert domain receptor
FosB	FBJ murine osteosarcoma viral oncogene homolog B
FPG	formamidopyrimidine DNA glycosylase
Fra-1	fos related antigen 1

Fra-2	fos related antigen 2
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GGR	global genome repair
GRX	glutaredoxin
GSH	glutathione
GSSG	glutathione disulfide
GTPase	Guanosine-5′-triphosphate
HCT116	Human colorectal carcinoma cell line 116
HEK293	Human embryonic kidney 293 cells
HeLa	Henrietta Lacks cells
HIF-1	hypoxia-inducible factor-1
HIF-1α	hypoxia-inducible factor-1 alpha
HR	homologous recombination
HRE	hypoxia response elements
HR32B	human homologue of the yeast RAD23 protein
HUVES	human umbilical vein endothelial cells
IL-2	interleukin 2
LP-BER	long-patch base excision repair
Lys6	lysine 2
Lys7	lysine 7
MeSeH	methylselenol
MGMT	O6-methylguanine-DNA methyltransferase
MiTF	Microphthalmia-associated transcription factor
MLH1	MutL homolog 1
MMR	mismatch repair
MMS	methyl methane sulfonate
MPG	N-methylpurine DNA glycosylase
MRN	Mre11/Rad50/Nbs1
MSH2	MutS homolog 2
MSH3	MutS homolog 3
MSH6	MutS homolog 6
MSI	microsatellite instability
MYH	MutY homolog
NAC	N-acetyl-L-cysteine
NADPH	Nicotinamide adenine dinucleotide phosphate
Nbs1	Nijmegen breakage syndrome 1

NEIL	fgp/nei family DNA glycosylase
NER	nucleotide excision repair
NF-κB	nuclear factor kappa B
NHEJ	non-homologous end joining
NK	natural killer
NTH	homolog of E. coli endonuclease III (nth)
O6-meG	O6 methyl guanine
OGG1	oxoguanine glycosylase 1
P2Y	purinergic receptor
PARP1	poly ADP ribose polymerase 1
PCEC	pancreatic cancer-associated endothelial cells
PCNA	proliferating cell nuclear antigen
PEBP-2	phosphatidylethanolamine-binding protein 2
PMS2	postmeiotic segregation increased 2
PRX	peroxiredoxin
Pol β	DNA polymerase beta
Pol δ	DNA polymerase delta
Pol ε	DNA polymerase epsilon
PMS1	post-meiotic-segregation increased-1 pro-tein
PMS2	post-meiotic-segregation increased-2 protein
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
Ref-1	redox effector factor 1
RF-C	replication factor C rRNA, ribosomal RNA
RNA Pol II	RNA polymerase II
ROS	reactive oxygen species
RPA	replication protein A
RPC	retinal progenitor cell
RPE	retinal pigment epithelium
RVEC	Retinal vascular endothelial cell
SeMet	selenomethionine
SETMAR	SET domain and mariner transposase fusion
siRNA	small interfering RNA
SMUG1	mammalian 5-formyluracil DNA glycosylase
SP-BER	short-patch base excision repair
ssDNA	single-strand DNA

TCR	transcription-coupled repair
TDG	thymine-DNA glycosylase
TFIIH	transcription factor IIH
TNF-α	tumor necrosis factor alpha
Trx	thioredoxin
TrxR	thioredoxin reductase
TSH	thyroid stimulating hormone
TTF-1	thyroid transcription factor 1
UNG	uracil-DNA glycosylase
UV-DDB	UV-damaged DNA binding protein
WRN	Werner protein, deficient in Werner's syndrome
XPB	xeroderma pigmentosum complementary group B protein
XPC	xeroderma pigmentosum complementary group C protein
XPD	xeroderma pigmentosum complementary group D protein
XPF1	xeroderma pigmentosum complementary group F protein
XPG	xerodermapigmentosum group
XRCC1	X-ray repair complementing defective re-pair in Chinese hamster cells 1
XRCC4	X-ray repair complementing defective repair in Chinese hamster cells 4

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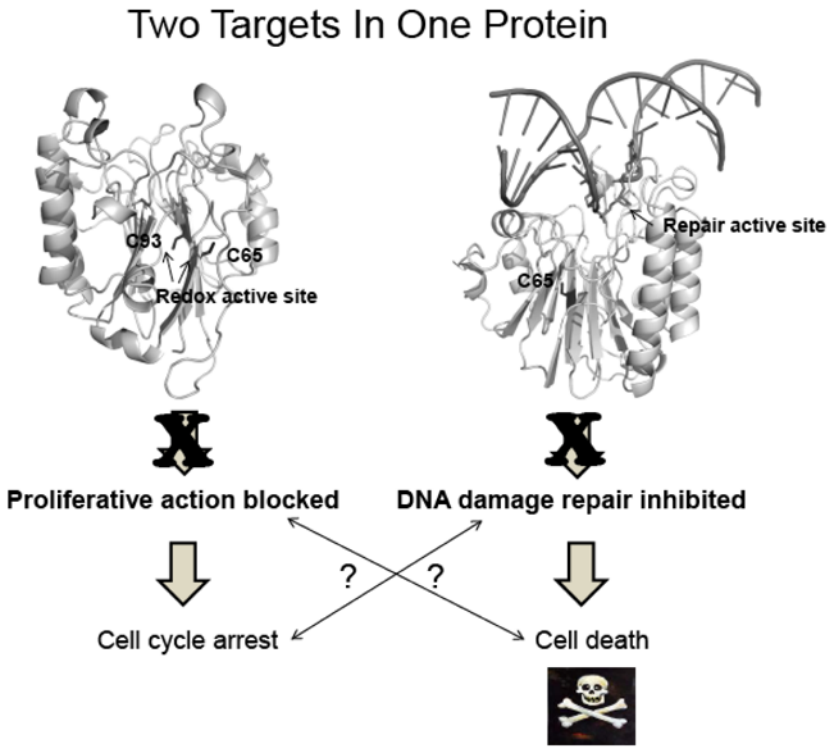

Fig. 1.

Two targets in one protein. APE1 has both redox and repair activities that involve distinct sites and residues in the protein. On the left panel is a ribbon rendering of APE1 in gray with the two Cys residues implicated in the redox activity, C65 and C93, shown in black sticks. On the right panel is a similar rendering of APE1 with a DNA substrate shown as a cartoon rendering in dark gray with abasic site and adjacent bases shown in dark gray sticks. The repair active site specifically recognizes duplex DNA containing an abasic site. Functional activities associated with redox or repair activities are shown along with the expected consequences of inhibition of these functions.

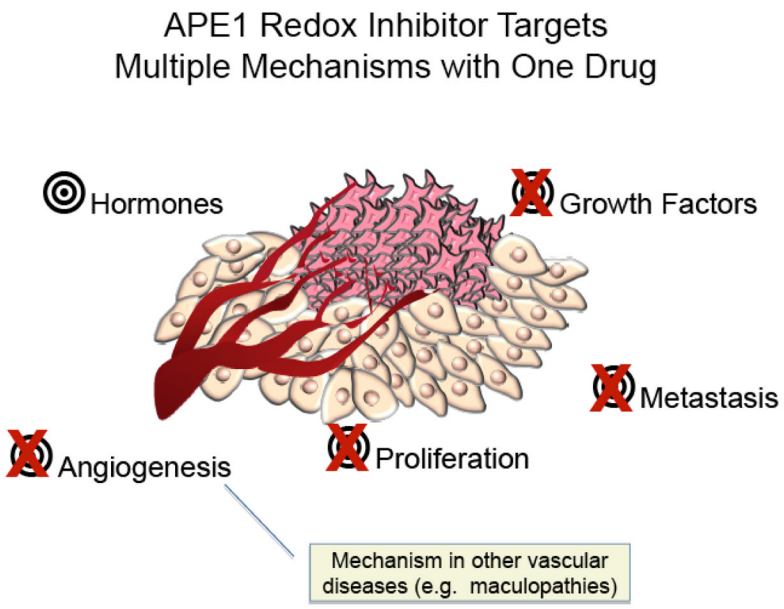

Fig. 2.

Inhibition of APE1 redox mechanism affects multiple cancer pathway targets. Blocking APE1 redox signaling affects various downstream targets that can impact on tumor growth factors, angiogenesis, growth and proliferation and metastasis and migration of the tumor.

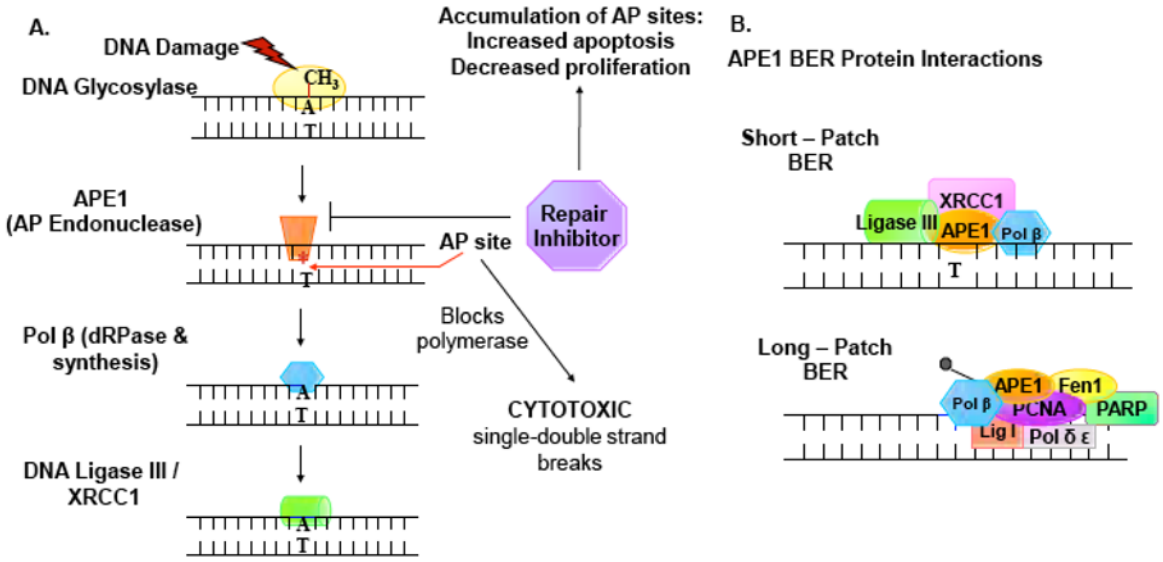

Fig. 3.

APE1 role in DNA base excision repair (BER). This figure gives a very general overview of the BER pathway and is discussed in more detail in other chapters in this review issue. **A**) Repair is initiated by a damage specific DNA glycosylase, which removes the damaged base to generate an AP site. Monofunctional DNA glycosylases remove the damaged base to generate an AP site (shown) whereas bifunctional glycosylases in addition to excising the damaged base also nick the phosphodiester backbone, 3′ to the AP site (not shown). APE1 processes the AP sites by hydrolyzing the backbone 5′ to the AP site to generate 3′OH and 5′ dRP groups. The 5′ dRP group is removed by the dRPase function of DNA Polymerase β and it also fills in the correct base. Repair is completed by DNA Ligase III/XRCC1 by sealing the nick. Failure to repair such accumulated AP sites leads to cytotoxicity, increased apoptosis. Elevated levels of APE1 in cancer cells have been linked to resistance to chemotherapy, poor prognosis and survival. Thus inhibiting APE1 leads to sensitization of cancer cells to chemotherapeutic agents [26].

B) In the major short-patch (SP) BER pathway regular AP sites are repaired by the removal of the single damaged base. Modified or oxidized AP sites are repaired by the long-patch (LP) BER pathway where a flap of 3–8 nucleotides is displaced and excised by Fen1. DNA polymerase β, δ or ε then inserts the correct nucleotides and repair is completed by sealing of the nick by DNA ligase I. APE1 also interacts with PCNA in the LP BER pathway [26].

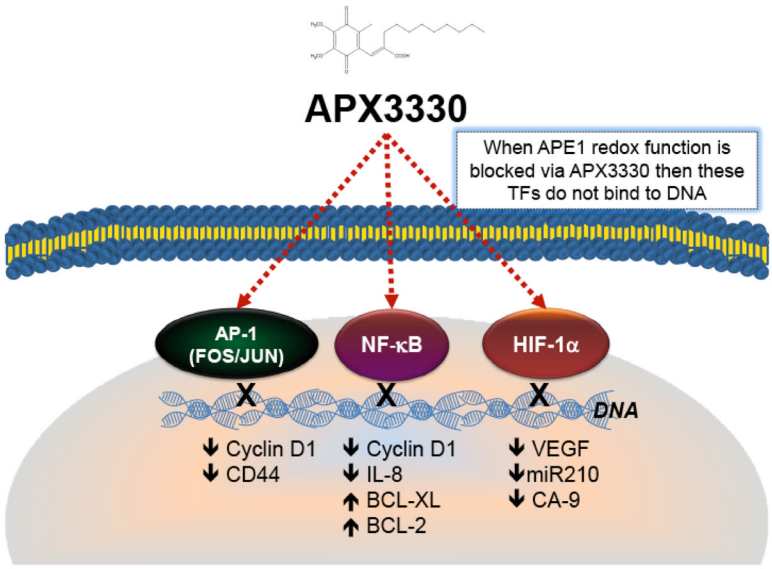

Fig. 4.

Inhibition of APE1 redox signaling is predicted to inhibit downstream disease-specific genes of the TFs it regulates. In this figure, three of seven identified relevant TFs for pancreatic cancer are shown [53]. All are targets of APE1 redox signaling and inhibiting this function should lead to altered responses [25, 27].

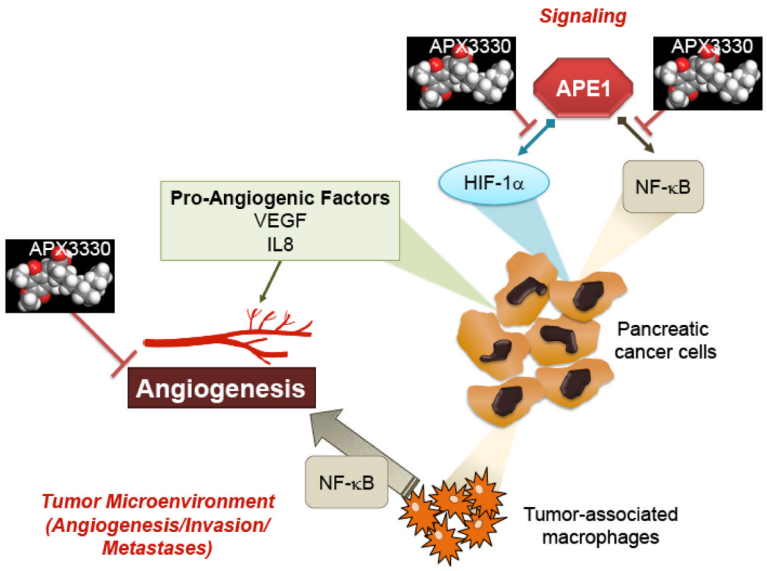

Fig. 5.

Role of APE1 on tumor microenvironment. As an example, blocking APE1 function could lead to a variety of alterations not only in the tumor, but also in the tumor microenvironment including decreased angiogenesis, inhibition of NFkB associated with macrophages and inflammation as well as the TF targets and effects in the tumor [53]. The redox inhibitor APX3330 and analogs have the potential to block all of these various aspects of APE1 signaling pathways in tumors and the surrounding microenvironment [24, 25, 27, 107–109, 116].

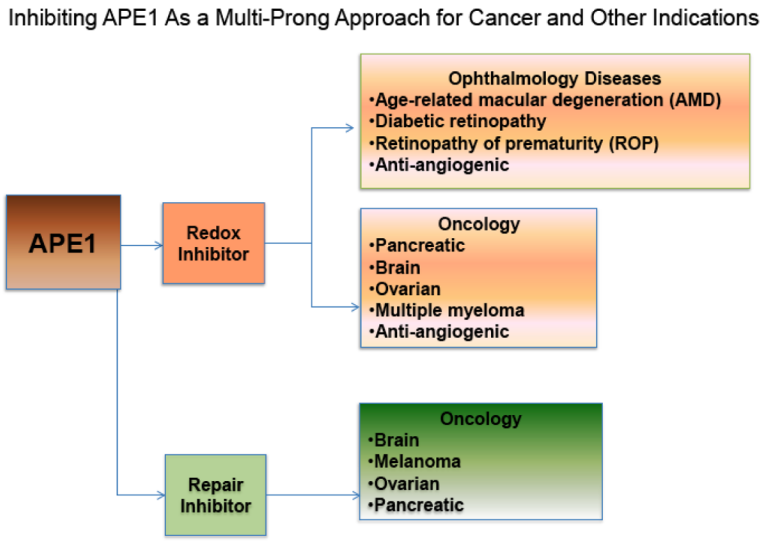

Fig. 6.

Inhibiting APE1 as a multi-prong approach for cancer and other indications. Blocking APE1 redox and DNA repair functions should provide multiple approaches for small molecule inhibitors of these functions in a variety of cancer and other indications. With the recent data supporting a role of APE1 in angiogenesis [25, 27, 107–111], a wide variety of indications such as AMD, ROP, diabetic retinopathy and others are potential disease areas for APE1 redox inhibition. APE1 DNA repair inhibition is more targeted toward cancer uses alone or with chemotherapeutic agents and ionizing radiation [37, 114, 121, 168].

Table 1

Transcription Factors Associated with DNA Repair Genes Potentially Regulated by APE1 Redox Activity

Transcription Factor Targets for APE1 Redox Signaling	Direct Repair	Base Excision Repair	Global Genome Repair (Nucleotide Excision Repair)	Mismatch Repair	Homologous Recombination
NF-κB	MGMT				
AP-1		UNG2	ERCC1 XPA RAD23B ERCC3	MLH1 MSH2 PMS2 MSH6	ATM RAD50
CREB		Polβ			
p53	MGMT	Polβ APE1 AAG	XPC DDB2	MLH1 MSH2 PCNA PMS2	RAD51 WRN
HIF-1α				MSH2 MSH6	NBS1

Transcription factors listed in the table are known to be regulated by APE1 redox signaling. These TFs have been shown to be involved in regulating a variety of DNA repair genes. Inhibition of APE1 redox function has the potential to affect DNA repair and stress response through blocking of these DNA repair genes.